

What is claimed:

1. A method to produce N-glucosamine by fermentation, comprising:

(a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism having a genetic modification in an amino sugar metabolic pathway, said amino sugar metabolic pathway selected from the group consisting of a pathway for converting N-glucosamine-6-phosphate into another compound, a pathway for synthesizing N-glucosamine-6-phosphate, a pathway for transport of N-glucosamine or N-glucosamine-6-phosphate out of said microorganism, a pathway for transport of N-glucosamine into said microorganism, and a pathway which competes for substrates involved in the production of N-glucosamine-6-phosphate;

wherein said step of culturing produces a product selected from the group consisting of N-glucosamine-6-phosphate and N-glucosamine from said microorganism; and

(b) recovering said product.

2. The method of Claim 1, wherein said N-glucosamine-6-phosphate is intracellular and said N-glucosamine is extracellular, wherein said step of recovering comprises a recovering step selected from the group consisting of recovering said N-glucosamine-6-phosphate from said microorganism, recovering said N-glucosamine from said fermentation medium, and a combination thereof.

3. The method of Claim 1, wherein said product is N-glucosamine which is secreted into said fermentation medium by said microorganism and wherein said step of recovering comprises purification of said N-glucosamine from said fermentation medium.

4. The method of Claim 1, wherein said product is intracellular N-glucosamine-6-phosphate and said step of

recovering comprises isolating said N-glucosamine-6-phosphate from said microorganism.

5. The method of Claim 1, wherein said product is intracellular N-glucosamine-6-phosphate and said step of recovering further comprises dephosphorylating said N-glucosamine-6-phosphate to produce N-glucosamine.

10. The method of Claim 1, wherein said step of culturing comprises maintaining said source of carbon at a concentration of from about 0.5% to about 5% in said fermentation medium.

15. The method of Claim 1, wherein said genetic modification is in a gene encoding a protein selected from the group consisting of N-acetylglucosamine-6-phosphate deacetylase, N-glucosamine-6-phosphate deaminase, N-acetyl-glucosamine-specific enzyme II<sup>Nag</sup>, N-glucosamine-6-phosphate synthase, phosphoglucosamine mutase, N-glucosamine-1-phosphate acetyltransferase-N-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II<sup>Glc</sup> of the PEP:glucose PTS, EIIM,P/III<sup>Man</sup> of the PEP:mannose PTS, and alkaline phosphatase.

20. The method of Claim 1, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule encoding N-glucosamine-6-phosphate synthase to increase expression of said N-glucosamine-6-phosphate synthase by said microorganism, wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence.

25. The method of Claim 8, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

30. The method of Claim 8, wherein said recombinant nucleic acid molecule encoding N-glucosamine-6-phosphate synthase comprises a genetic modification which reduces N-

glucosamine-6-phosphate product inhibition of said N-glucosamine-6-phosphate synthase.

11. The method of Claim 8, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of N-acetylglucosamine-6-phosphate deacetylase, N-glucosamine-6-phosphate deaminase, N-acetyl-glucosamine-specific enzyme II<sup>Nag</sup>, phosphoglucosamine mutase, N-glucosamine-1-phosphate acetyltransferase-N-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II<sup>Glc</sup> of the PEP:glucose PTS, EIIM, P/III<sup>Man</sup> of the PEP:mannose PTS, and alkaline phosphatase, wherein said genetic modification decreases enzymatic activity of said protein.

12. The method of Claim 8, wherein said microorganism has a modification in genes encoding N-acetylglucosamine-6-phosphate deacetylase, N-glucosamine-6-phosphate deaminase and N-acetyl-glucosamine-specific enzyme II<sup>Nag</sup>, wherein said genetic modification decreases enzymatic activity of said protein.

13. The method of Claim 12, wherein said genetic modification is a deletion of at least a portion of said genes.

14. The method of Claim 1, wherein said microorganism is selected from the group consisting of bacteria and yeast.

15. The method of Claim 1, wherein said microorganism is a bacterium of the genus *Escherichia*.

16. The method of Claim 1, wherein said microorganism is *Escherichia coli*.

17. The method of Claim 16, wherein said genetic modification is a mutation in an *Escherichia coli* gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glmS*, *ptsG* and alkaline phosphatase gene.

18. A method to produce *N*-glucosamine by fermentation, comprising:

(a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, an *Escherichia coli* transformed with a recombinant nucleic acid molecule encoding *N*-glucosamine-6-phosphate synthase, wherein said recombinant nucleic acid molecule increases expression of said *N*-glucosamine-6-phosphate synthase by said *Escherichia coli*, and wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence;

wherein said step of culturing produces a product selected from the group consisting of *N*-glucosamine-6-phosphate and *N*-glucosamine from said *Escherichia coli*; and

(b) recovering said product.

19. The method of Claim 18, wherein said recombinant nucleic acid molecule comprises a genetic modification which reduces *N*-glucosamine-6-phosphate product inhibition of said *N*-glucosamine-6-phosphate synthase.

20. The method of Claim 18, wherein said *Escherichia coli* has an additional genetic modification in at least one gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glms*, *ptsG* and alkaline phosphatase gene.

25. The method of Claim 18, wherein said *N*-glucosamine-6-phosphate is intracellular and said *N*-glucosamine is extracellular, wherein said step of recovering comprises a recovering step selected from the group consisting of recovering said *N*-glucosamine-6-phosphate from said microorganism, recovering said *N*-glucosamine from said fermentation medium, and a combination thereof.

22. A microorganism for producing N-glucosamine by a biosynthetic process, said microorganism being transformed with a recombinant nucleic acid molecule encoding N-glucosamine-6-phosphate synthase, said recombinant nucleic acid molecule being operatively linked to a transcription control sequence and comprising a genetic modification which reduces N-glucosamine-6-phosphate product inhibition of said N-glucosamine-6-phosphate synthase;

wherein expression of said recombinant nucleic acid molecule increases expression of said N-glucosamine-6-phosphate synthase by said microorganism.

23. The microorganism of Claim 22, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

24. The microorganism of Claim 22, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of N-acetylglucosamine-6-phosphate deacetylase, N-glucosamine-6-phosphate deaminase, N-acetylglucosamine-specific enzyme II<sup>Nag</sup>, phosphoglucosamine mutase, N-glucosamine-1-phosphate acetyltransferase-N-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II<sup>Glc</sup> of the PEP:glucose PTS, EIIM,P/III<sup>Man</sup> of the PEP:mannose PTS, and alkaline phosphatase, wherein said genetic modification decreases enzymatic activity of said protein.

25. The microorganism of Claim 22, wherein said microorganism has a modification in genes encoding N-acetylglucosamine-6-phosphate deacetylase, N-glucosamine-6-phosphate deaminase and N-acetyl-glucosamine-specific enzyme II<sup>Nag</sup>, wherein said genetic modification decreases enzymatic activity of said protein.

26. The microorganism of Claim 25, wherein said genetic modification is a deletion of at least a portion of said genes.

5 27. The microorganism of Claim 22, wherein said microorganism is selected from the group consisting of a yeast and a bacterium.

28. The microorganism of Claim 22, wherein said microorganism is a bacterium of the genus *Escherichia*.

10 29. The microorganism of Claim 22, wherein said microorganism is *Escherichia coli*.

15 30. The microorganism of Claim 29, wherein said *Escherichia coli* has at least one additional genetic modification in a gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *ptsG* and alkaline phosphatase gene, wherein said genetic modification decreases enzymatic activity of a protein encoded by said gene.

20 31. The microorganism of Claim 29, wherein said *Escherichia coli* has a deletion of *nag* regulon genes.

25 32. The microorganism of Claim 29, wherein said *Escherichia coli* has a deletion of *nag* regulon genes and a genetic modification in *manXYZ* genes such that the proteins encoded by said *manXYZ* genes have decreased enzymatic activity.

25 33. The microorganism of Claim 22, wherein said microorganism produces at least about 1 g/L of N-glucosamine when cultured for about 24 hours at 37°C to a cell density of at least about 8 g/L by dry cell weight, in a pH 7.0 fermentation medium comprising: 14 g/L  $K_2HPO_4$ , 16 g/L  $KH_2PO_4$ , 1 g/L  $Na_3Citrate \cdot 2H_2O$ , 5 g/L  $(NH_4)_2SO_4$ , 20 g/L glucose, 10 mM  $MgSO_4$ , 1 mM  $CaCl_2$ , and 1 mM IPTG.

34. A microorganism for producing *N*-glucosamine by a biosynthetic process, said microorganism comprising:

(a) a recombinant nucleic acid molecule encoding *N*-glucosamine-6-phosphate synthase, said recombinant nucleic acid molecule being operatively linked to a transcription control sequence, wherein expression of said recombinant nucleic acid molecule increases expression of said *N*-glucosamine-6-phosphate synthase by said microorganism; and,

(b) at least one genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, *N*-glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II<sup>Nag</sup>, phosphoglucosamine mutase, *N*-glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II<sup>Glc</sup> of the PEP:glucose PTS, EIIM,P/III<sup>Man</sup> of the PEP:mannose PTS, and alkaline phosphatase, wherein said genetic modification decreases enzymatic activity of said protein.

35. The microorganism of Claim 34, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

36. The microorganism of Claim 34, wherein said microorganism is selected from the group consisting of a yeast and a bacterium.

37. The microorganism of Claim 34, wherein said microorganism is a bacterium of the genus *Escherichia*.

38. The microorganism of Claim 34, wherein said microorganism is *Escherichia coli*.

39. The microorganism of Claim 34, wherein said microorganism produces at least about 1 g/L of *N*-glucosamine when cultured for about 24 hours at 37°C to a cell density of at least about 8 g/L by dry cell weight, in a pH 7.0 fermentation medium comprising: 14 g/L K<sub>2</sub>HPO<sub>4</sub>, 16

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g/L  $\text{KH}_2\text{PO}_4$ , 1 g/L  $\text{Na}_3\text{Citrate} \cdot 2\text{H}_2\text{O}$ , 5 g/L  $(\text{NH}_4)_2\text{SO}_4$ , 20 g/L glucose, 10 mM  $\text{MgSO}_4$ , 1 mM  $\text{CaCl}_2$ , and 1 mM IPTG.

Add air